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The Porcine Eye as a Surrogate Model for the Human Eye: Anatomical and Mechanical Relationships

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ABSTRACT

Although the porcine eye has been used as an experimental model for the human eye for decades, this substitution has never been thoroughly justified. Based on experimental results with porcine and human eyes, this study utilized a two part approach to compare the anatomical and mechanical differences between species. Part one quantified the anatomical differences. Macroscopically, the two eyes are comparable spheres with a 24 mm diameter. The sclera is thicker in the human, which ranges from 0.30 mm to 1.35 mm, compared to a range of 0.25 mm to 0.90 mm in the porcine. Microscopically, the cellular makeup of the cornea, ciliary body, and retina are nearly identical. Part two examined the material properties of the cornea and sclera for each species. Using quasistatic one dimensional tensile tests, the modulus of elasticity for both the human and porcine eyes was shown to be highly sensitive to loading rate; however, the lack of comparative experiments at large strains limited the mechanical comparison. Due to its similar size, structure, and its availability for use relative to time of death, the porcine eye is recommended as a better model than the postmortem human eye for predicting injury.

INTRODUCTION

Over 2.4 million eye injuries occur each year as a result of trauma in the United States alone (Parver, 1986). Of these patients, 40,000 are left with significant visual impairment, and over 30,000 of these are left blind in at least one eye (Lueder, 2000). Severe eye injuries, such as hyphemas and ruptured globes, are often the result of impact from sports equipment or contact with an airbag or steering wheel in automobile crashes (Vinger *et al.*, 1999, and Duma *et al*, 1996). In order to investigate these types of injuries, researchers have used a range of eye tissues. Although the human eye seems the logical experimental tool, it is not typically a feasible research tool for two reasons. First, most healthy eyes are used for tissue transplants and thus are not suitable for experimental use. Second, the supply of human eyes is too low and the delay in getting them too great to be regularly used. By the time a human eye can be used for experimental purposes, the delay can range from one to eight days postmortem. In this timeframe the eye experiences severe tissue autolysis that negates its usefulness in injury evaluation research.

The porcine eye has been used extensively in eye injury research and has emerged as a readily available surrogate for the human eye; however, this difference between the human and porcine eye have not previously been investigated. Two early research studies on eye injuries utilized *in vitro* porcine eyes to evaluate the risk of eye injury to BB impacts. (Delori, 1969, and Weidenthal, 1966). Additional studies to evaluate the risk of eye injuries in an automobile environment utilized porcine eyes subjected to air bag deployments (Fukagawa *et al.*, 1993, and Duma *et al.*, 1997). Two recent studies used kinetic energy of impacting objects with porcine eyes to evaluate the risk of eye injury (Scott *et al.*, 2000, and Duma *et al.*, 2000).

The purpose of this paper is to investigate the validity of substituting the porcine eye for the human eye by quantifying the similarities and differences between the two. A two part investigation is presented that first compares the anatomical properties and second compares the mechanical properties.

BACKGROUND

ANATOMY REVIEW – The outer protective layer of the human and porcine eye is made up of the sclera and the cornea, which comprise approximately 85% and 15% of the surface area respectively (Figure 1). The anterior chamber is located between the cornea and the iris/lens, and its watery fluid is filtered and replaced hourly. The shape of the lens is controlled by the ciliary muscle in order to properly focus light on the retina. The large volume in the center of the eye is filled with the vitreous humor, which is a

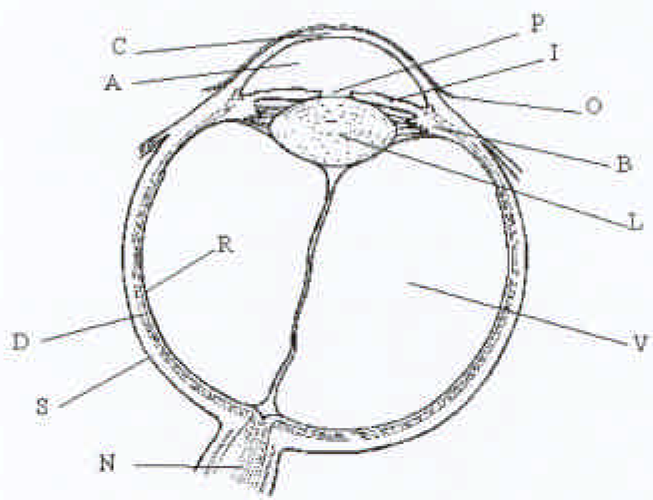

Figure 1. Primary ocular structures: A = anterior chamber, B = ciliary body, C = cornea, D = choroid, I = iris, L = lens, N = optic nerve, O = conjunctiva, P = pupil, R = retina, S = sclera, V = vitreous humor.

gelatin-like substance that, unlike the fluid in the anterior chamber, does not circulate and filter. The posterior 85% consists of three layers, the outermost being the sclera which forms into the cornea anteriorly. The middle layer is the choroid, which becomes the ciliary body and iris anteriorly, and is responsible for providing the blood supply to the retina. The retina, the delicate innermost layer, is the light transducing portion of the eye.

METHODS

The analysis consisted of a two part investigation. Part one comprised an anatomical comparison that was performed at the macroscopic and microscopic levels. First, the macroscopic data was analyzed from published studies. Second, in order to get a more detailed comparison, a microscopic analysis was performed. Three key locations relating to injury potential were investigated: the anterior portion of the cornea, the thinnest portion of the equator, and the limbus, or intersection between the cornea and sclera. Human eyes were obtained from the Old Dominion Eye Foundation (Richmond, VA). Samples were from a Caucasian Male, age 69, and a Black Female, age 70. The human eyes were enucleated within seven hours postmortem and stored in saline temporarily wrapped in gauze, then were fixed and stored in 10% buffered formalin. Porcine eyes were obtained from a local abattoir. The pigs used were Yorkshires, aged between six and seven months, weighing approximately 225 lbs, an age and weight corresponding to a fully developed porcine animal. Eyes were enucleated within 15 minutes postmortem and fixed in 10% buffered formalin.

The same processing techniques were used for both the human and porcine eyes. Next, both the human and porcine eyes were embedded in paraffin and sectioned with a microtome into 6 to 8 micron thick slices for slide mounting. Two sagittal sections, along a vertical sagittal plane perpendicular to the long posterior ciliary arteries were used for each eye. Sections were then stained with periodic acid-Schiff (PAS) and Hematoxylin and Eosin (H&E). Slides were then examined under a microscope at 10X to obtain a cross sectional view of the thickness through the cornea and sclera. A view of the limbus, including the ciliary body, and corneoscleral junction, was also obtained for both the human and porcine specimens.

Part two involved a comparison of the mechanical properties of the human and porcine eyes. This data was gathered from previously published experiments and organized by similar testing methodologies.

RESULTS

Part 1: Anatomical Comparison

Macroscopically, the porcine globe is slightly oval with medial-lateral, superior-inferior, and anterior-posterior diameters of 25.5, 24.5, and 21.6 mm, respectively (Table 1 and Figure 2). (Bartholomew *et al.*, 1997; Duke-Elders *et al.*, 1961; Prince 1960). Gross ocular dimensions vary greatly with age in the pig and there is likely considerable breed variation (Bartholomew, 1997). The human globe is more spherical with respective diameters of 24, 23, and 24 mm. In general, the size of the eye is remarkably similar between humans and pigs. Globe volumes are reported to be 7 ml and 6-6.5 ml for human beings and pigs, respectively (McMenahin, 1991) The porcine orbit is conical in shape with respective diameters of 34, 43, and 61 mm. The human orbit is similarly shaped, with respective diameters of 40, 35, and 58 mm. The porcine eye is oriented rostrolaterally, with its visual axis oriented approximately 35° from midline, whereas the visual axis of the human eye is oriented more or less directly rostral with little to no deviation from midline (Prince, 1960). The bony orbit is complete in man. In the pig, the orbit is open and continuous with the temporal fossa. There is considerable variation in the orbit between different porcine breeds (Vestre, 1986). Similar to human beings, the porcine orbit contains large amounts of extraconal fat (Brooks, *et al.*, 1998). The pig has a nictitating membrane located in the intranasal orbit. This structure is vestigial in most primates, including humans. In both species, there are 4 rectus and 2 oblique extraocular muscles. Insertion points of these muscles are presented (Table 1). Retractor bulbi muscles are present in the pig and insert just posterior to the equator of the globe (Prince, 1960). In human beings, these muscles are vestigial structures (Duke-Elder, 1961).

Table 1. Porcine and Human Eye Average Structural Geometry.

Variable	Human Eye (mm)	Porcine Eye (mm)
globe medial-lateral, a-b	24	25.5
globe superior-inferior, c-d	23	24.5
globe anterior-posterior, e-f	24	21.6
orbit medial-lateral	40	34
orbit superior-inferior	35	43
orbit anterior-posterior	58	61
Cornea medial-lateral, g-h	11.7	16.6
Cornea superior-inferior, i-j	10.6	14
Cornea thickness at apex[1], k	0.7(0.5)	0.98
Cornea thickness at limbus[1], l	1.1(0.74)	1.2
Cornea radius at anterior surface, m	8	10.5
sclera thickness at limbus, n	0.83	0.55
sclera thickness at equator, o	0.45	0.25
sclera thickness at posterior pole, p	1.2	0.9
lens diameter	9	12.5
lens maximum thickness	4.5	9.2
superior rectus insertion from limbus, i-q	7.8	5.5
inferior rectus insertion from limbus, j-r	6.6	4.5
medial rectus insertion from limbus, h-s	5.5	8
Lateral rectus insertion from limbus, g-t	7	3.5
choroid thickness	0.15	0.14

[1]Corneal thickness is greater after death due to swelling. (In life) average thickness measurements not available in pigs.

The porcine cornea is larger and thicker than the human cornea. Both are horizontally slightly ovoid and both are thicker at the limbus than the apex. In histologic cross-section, the human and porcine corneas are similar with one notable exception. Both have an anterior stratified squamous epithelial layer, which is 5-7 cells thick in human beings (Pepose; 1992) and 6-8 cells thick in the pig (Prince 1960). In both species, the majority (approximately 90%) of the corneal thickness is comprised by a collagenous stroma. In humans and other primates, a 10-13 μm acellular membrane is located at the anterior aspect of the corneal stroma (Duke-Elder, 1961). Termed Bowman's membrane, it is considered part of the corneal stroma. This structure is not found in other mammals, including the pig. In both humans and pigs, the posterior surface of the cornea is lined by a monolayer of flattened, hexagonally shaped cells, termed the corneal endothelium. Corneal endothelial cell counts in the young human being are approximately 3500 cells/mm^2, with the cells being approximately 20 μm in diameter and 4-6 μm thick (Pepose; 1992).

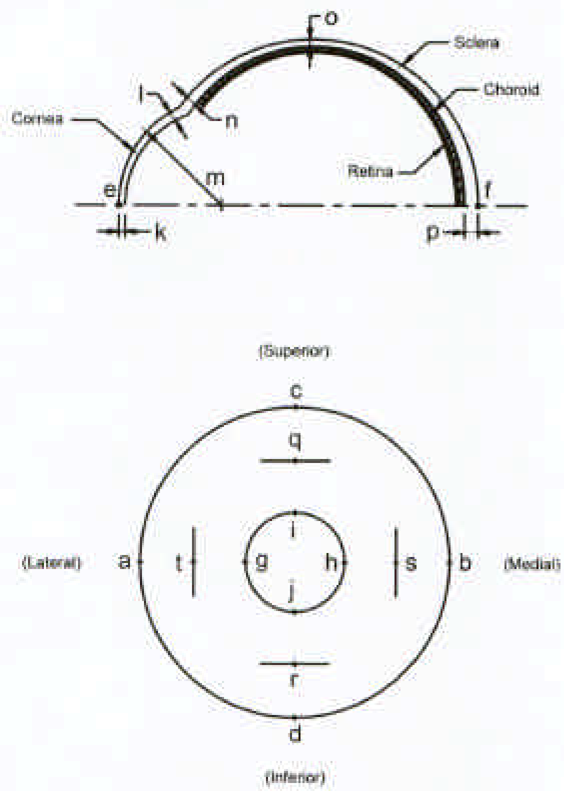

Figure 2. Dimension locations relative to Table 1.

Anterior chamber depth in the young adult pig, from the posterior cornea to the anterior surface of the lens has been reported as 2.47 mm (Bartholomew, *et al.* 1997). Anterior chamber depth in humans has been reported to range from 2.7 to 3.7 mm, with a decrease in depth with increasing age (Duke-Elder, 1961). Anterior chamber volumes are similar between pigs and human beings (McMenamin, 1991).

The iris of humans is thinnest at the root of the iris and thickest at the pupillary margin. In the pig the root of the iris is approximately twice as thick as that of the human, and iridal thickness tapers down to its narrowest point at the pupillary margin (Bartholomew, *et al.*; 1997; Kumar, 1990). When constricted, the pupil of the porcine eye is very slightly horizontally oval and assumes a circular shape when dilated (Vestre, 1986).

The ciliary body and aqueous humor outflow system in the pig differ from man in several respects. As in all reported nonprimate mammals, there is no single canal of Sclemm, but rather a series of aqueous collection vessels called the angular aqueous plexus (Tripathi, 1972). The ciliary body musculature in humans is well developed compared to the pig and other non-primate mammals (McMenamin, 1991). The porcine eye does, however, feature a shallow scleral sulcus containing a wedge-shaped mass of corneoscleral tissue comparable to the human trabecular meshwork (McMenamin, 1991).

The porcine lens is 12.5 mm in diameter with an axial thickness of 9.2 mm. The human lens is smaller, being 9 mm in diameter with an axial thickness of 4.5 mm. Geometrically, the two lenses are similar, with a steeper posterior than anterior curvature. The suspensory apparatus of the lens in both human beings in pigs is comprised of a 360° array of zonular fibers attaching the equatorial lens to the ciliary body. It has been stated that the zonular fibers in the pig are stronger than those in human beings, but documentation to support this statement could not be found in the literature (Kumar, 1990).

The human sclera is thicker, ranging from 0.45 to 1.2 mm, compared with 0.25 to 0.9 mm in the porcine. Both are thinnest at the equator and thickest at the posterior pole. Vitreous volume in both species is similar, being approximately 4 ml in the human eye and 3.5 ml in the porcine (Samuelson, 1999). The porcine retina is similar to the human in many respects (Beauchemin, 1974). The regions of the ora serrata and pars plana ciliaris are similar between both species (Weidenthal, 1966). Both humans and pigs possess a holangiotic retinal vascular pattern with a similarly sized capillary meshwork supplying identical layers of the retina (Rootman, 1971). While the porcine retina does not possess a true macula, a region of high cone concentration is present in the central retina. The porcine and human choroids are similar in their lack of a tapetum lucidum, a structure encountered in most other nonprimate mammalian species (Vestre, 1986). Average choroidal thickness is 0.15 mm in the human and 0.14 mm in the pig.

In the microscopic study of anatomical comparison, histologic cross sections of the cornea, sclera, and limbus were compared between a representative human eye sample and a porcine eye sample. Both samples show vacuolation and separation between layers of collagen in the corneas (Figure 3). Separation of layers is probably artifact due to processing the tissue. The greater thickness of the porcine cornea is also visible. At the thinnest portion of the equator, corresponding to the widest part of the eye in a frontal plane, the thinness of the porcine equator compared to the human is apparent (Figure 4). Detached retina can be seen to the left of the specimens, and layer separation within the thickness of the sclera can also be seen.

At the limbus, the demarcation between the cornea and sclera in the porcine eye is much more clearly stained (Figure 5). The appearance of the ciliary body between specimens of the same species and between the two species was variable, due to sectioning of the material. The ciliary bodies are highly nonuniform and therefore do not give consistent 2-D representations when they are sectioned. However, the arrangement and appearance of collagen in the slides is similar. The cornea increases in thickness abruptly in the porcine eye at the intersection between the cornea and sclera, forming a 'bulge' apparent in all slides examined.

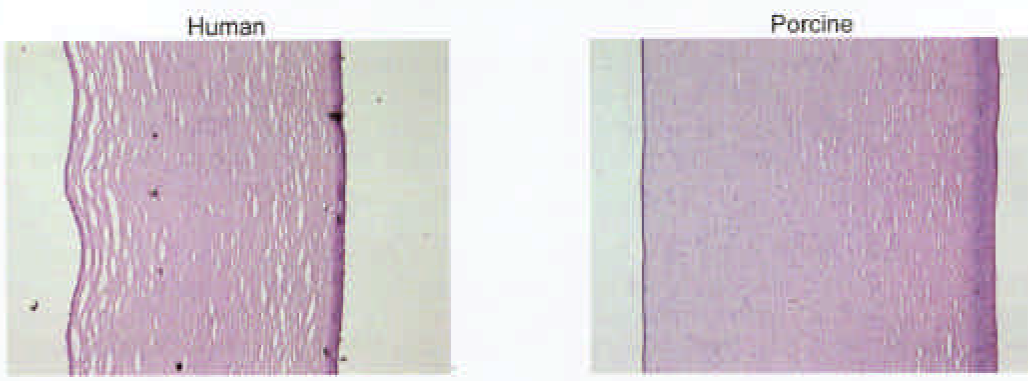

Figure 3. Human and porcine cornea anatomical cross sections (10X).

Figure 4. Human and porcine equator (sclera) anatomical cross sections (10X).

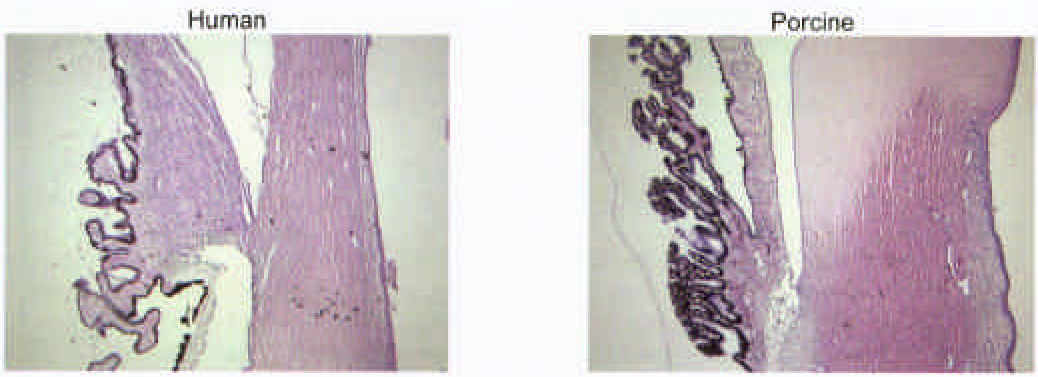

Figure 5. Human and porcine limbus anatomical cross sections (5X).

Part 2: Mechanical Comparison

Previous work has been done to determine the material properties of the human sclera and cornea (Andreassen *et al.*, 1980; Battaglioli *et al.*, 1984; Bryant *et al.*, 1994; Hanna *et al.*, 1989; Hoetzel *et al.*, 1992; Nash *et al.*, 1982; Shin *et al.*, 1997; Uchio *et al.*, 1999; Woo *et al.*, 1972; Yamada, 1970). The methods used have been either uniaxial strip extensiometry or membrane inflation. The results showed that the human sclera and cornea exhibit non-linear material behavior, are anisotropic, and viscoelastic. Woo *et al.* (1972) used membrane inflation to determine an exponential relationship between effective stress and effective strain [1].

$$\sigma_{ef} = \alpha\left(e^{\beta\varepsilon_{ef}} - 1\right) \qquad [1]$$

Effective stress and effective strain relate to the average three-dimensional states of stress and strain [2,3].

$$\sigma_{ef} = \frac{1}{3}\left\{(\sigma_1 - \sigma_2)^2 + (\sigma_2 - \sigma_3)^2 + (\sigma_3 - \sigma_1)^2\right\}^{\frac{1}{2}} \qquad [2]$$

$$\varepsilon_{ef} = \frac{2}{3}\left\{(\varepsilon_1 - \varepsilon_2)^2 + (\varepsilon_2 - \varepsilon_3)^2 + (\varepsilon_3 - \varepsilon_1)^2\right\}^{\frac{1}{2}} \qquad [3]$$

where $\sigma_1, \sigma_2, \sigma_3$, and $\varepsilon_1, \varepsilon_2, \varepsilon_3$ are the principal stress and strain components, respectively.

The coefficients (α,β) for the sclera and cornea were equal to ($1.8*10^4$,41.8) and ($5.4*10^3$,28.0) Pa, respectively. These tests involved measuring changes in displacement at the anterior and posterior poles only. The cornea and sclera were experimentally determined to be isotropic at these poles. However, isotropic behavior may not hold at other locations. Shin *et al.* (1997) also used membrane inflation, but measured displacements at several locations in human corneas. The cornea was determined to be anisotropic from these experiments. Evidence that random collagen fiber orientation at the corneal apex gives way to a circumferential orientation at the corneal limbus may be responsible (Meek *et al.*, 1982, 1987).

Battaglioli and Kamm (1984) conducted tests on strips of human sclera samples to determine the mechanical properties of the tissue in compression. Compressive stress-strain curves of the samples were found to be approximately linear, with an average modulus of $3.34\text{x}10^4$ Pa. This elastic modulus for compressive strength across the thickness was found to be approximately 100 times less than the modulus for tensile strength in the tangential direction. This can be explained by the fact that layers of collagen fibers run tangentially throughout the sclera but not radially (Duke-Elders *et al.*, 1961).

Membrane inflation has two advantages compared to single axis strip tests for the comparison of mechanical properties: membrane inflation preserves the globe geometry and collagen fiber integrity, and membrane inflation provides for bi-axial loading more similar to the *in vivo* loading condition. However, no membrane inflation or bi-axial test data exists for the porcine eye tissues. Single axis strip tests of human and porcine eye tissues are more common and can be used to adequately compare the behaviors of different species (Hoeltzel, 1992). Andreassen (1980), Bryant (1994), Hoeltzel (1992), and Uchio (1999) determined the stress strain curves of human corneal strips under uniaxial tension (Figure 6). Andreassen (1980) used a constant deformation rate of 1 cm/min up to failure. Bryant (1994) applied a rate of 0.6 cm/min to half of the samples, and 3 cm/min for the remaining half, also up to failure. The stress strain curve represents an average of all the samples. Hoeltzel (1992) applied a 0.05 cm/min deformation rate, not up to failure. Uchio (1999) tested both corneal and scleral strips up to rupture but did not report the deformation rate used. Only the curve for the cornea is shown since no tests have been done with porcine scleras to use as a comparison.

Kampmeier (2000) applied a deformation rate of 0.08 cm/min to porcine corneal strips, not up to rupture (Figure 6). The strips were extended at different angular orientations and the results illustrated anisotropic behavior. Nyquist (1968) performed strip tests with porcine corneas under three constant creep loads. Instantaneous and steady state strains were recorded at each of the three loads. Instantaneous and steady state stress strain curves were then curve-fitted to each respective pair of three data points, a statistically small number of samples. In addition, quantifiable descriptions were not given for how instantaneous and when steady state was reached. Comparing the stress strain curves of the two species, both exhibit non-linear, viscoelastic (rate dependent) behavior. Research with porcine eyes is too limited at this time to provide a quantitative comparison of the porcine and human eye material properties. Tests with both species, for both the cornea and the sclera, up to rupture, under identical test conditions, such as temperature, age, postmortem time, loading rate, orientation and location of samples, need to be conducted for a more exact comparison.

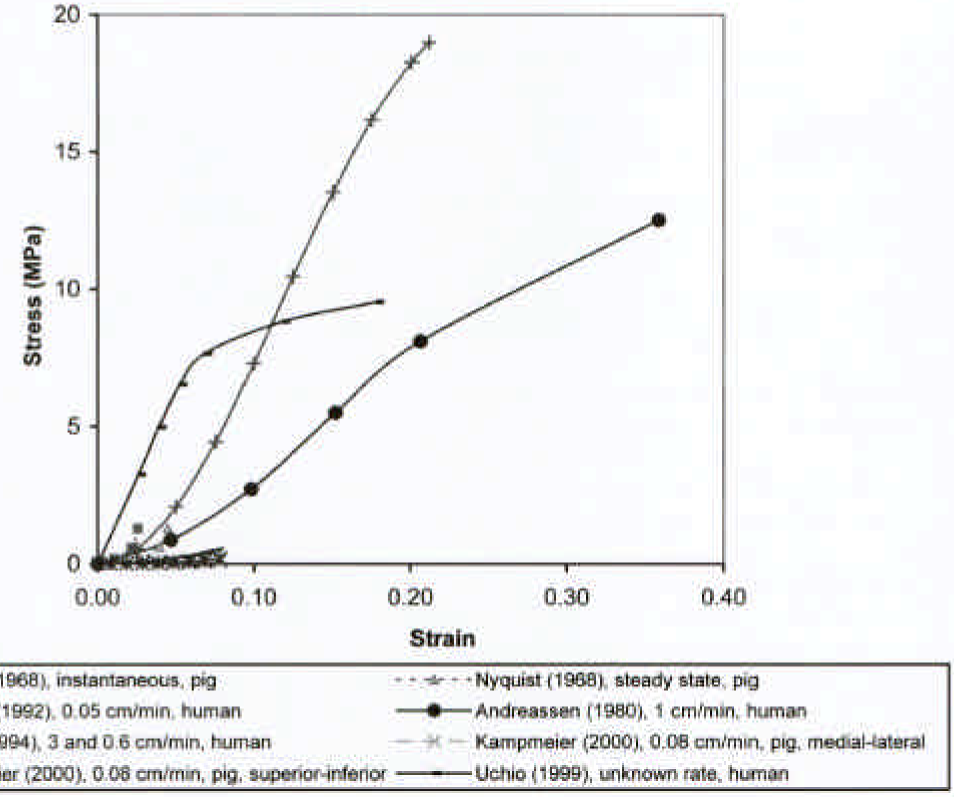

Figure 6. Stress strain curves of human and porcine cornea strips.

CONCLUSIONS

While there are considerable differences between the porcine and human orbits and eye orientation, these differences are lessened when only the globe is considered. Anatomically, the porcine eye is very similar to the human eye. The porcine eye has the same macroscopic anatomical features with only small differences apparent at the microscopic level. The volumes and diameters (medial-lateral, superior-inferior, and anterior-posterior) of human and porcine eyes are similar. Although there are slight differences in the thicknesses of the cornea and sclera in the porcine and human models, it is not anticipated that these small differences would change the injury outcome. At present, there is insufficient data with relevant strain rates and ranges needed for injury analysis to compare the human and porcine eyes. An important contribution that porcine eyes offer is their ready availability. Though the optimal solution would be to use human eyes from fresh cadavers, this solution is not viable. The benefit of using porcine eyes is the injury response evaluation that is possible with fresh tissue. For example, the fresh porcine cornea is capable of reproducing corneal abrasions better than human cornea that is typically only available from older individuals several days postmortem. In summary, the porcine eye appears to be a suitable surrogate for use in human eye injury research studies.

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